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Ref: Expert Report for the PhD Thesis Titled "Effect of Androgenic Hormones on Mesenchymal Stromal / Progenitor Cells Involved in Cardiovascular Regeneration" by Popa Mirel Adrian

Dear Dr. Simionescu:

Thank you for having me as an expert to review the PhD thesis work of Mr. Popa Mirel Adrian. I have looked through his work with great interest. Attached kindly find my final report regarding his work.

I hope my comments will be sufficient to support his candidacy and please feel free to contact me in case there are any concerns.

With best regards

Raghvendra K Dubey, PhD

Professor for Molecular Reproductive Endocrinology
Department for Reproductive Endocrinology
University Zurich
Switzerland

Expert Report for the Dissertation Titled **"Effect of Androgenic Hormones on Mesenchymal Stromal / Progenitor Cells Involved in Cardiovascular Regeneration"** submitted to **School of Advanced Studies of the Of the Romanian Cell Academy** and conducted at Institute of Biology and Pathology "Nicolae Simionescu" by **Popa Mirel Adrian.**

In the present thesis, Mr. Popa Mirel Adrian has attempted to investigate and define the modulatory actions of androgens in facilitating adult stem cell i.e. mesenchymal stromal and progenitor endothelial cell mediated cardiac repair. Use of adult stem cells, is postulated to be of great therapeutic significance in treating cardiac injury associated with heart disease. Although promising, the clinical use of this cell-based technology has been limited by poor integration of stem cells at the site of cardiac damage thereby limiting their repair potential. Therefore, a major challenge in this field is to investigate ways to improve adult stem cell integration. In this context, finding molecules that induce stem cell growth (proliferation, migration and adhesion) and potentiate the release of pro-growth and angiogenic autocrine/paracrine factors is of key importance. Since sex steroids, including androgens, are known to modulate growth and function of cardiovascular cells, they may potentiate the repair capability of adult stem cells. In the present doctoral work Popa has investigated the impact of androgens (Dihydrotestosterone; DHT and testosterone) on the growth and integration of Wharton's jelly derived MSCs in cardiac tissue, moreover, he has dissected the underlying mechanisms involved. Additionally, he has investigated modulatory effect of androgens on the integration of PECs in cardiac tissue.

To accomplish the goals for his thesis work Mr. Popa utilized multiple state of the art in vitro techniques, including isolation and characterization of MSCs from Wharton's jelly; co-culture non-contact set up of heart tissue slices and stem cells to assess their migration and integration; analytical and biochemical assays (Western Blotting, RT-PCR, ELISA, Luminex, Proteomic analysis; immunostaining, microscopy, xCELLigence live cell growth assay) to delineate the underlying mechanisms.

The overall key findings of his work can be divided in two parts. First he has demonstrated that androgens induce proliferation and migration of human Wharton's jelly MSCs, moreover, pretreatment with DHT significantly potentiates their directed migration and integration within the cardiac tissue. The major mechanism(s) driving the cascade of events resulting in increased integration involves DHT mediated modulation of MMPs in a spatiotemporal fashion, generation of VEGF and Angiogenin and modulation of androgen receptors as well as adhesion molecules. The convincing and detailed findings of his work were published in the peer reviewed Journal of Molecular Endocrinology.

In the second part of his work, yet unpublished, he assessed the impact of androgens on the proliferation, migration and integration of EPCs. Mr. Popa demonstrated that DHT induces capillary formation and these effects were androgen receptor mediated. Importantly, DHT activates key proteolytic enzymes (MMP-9, EMMPRIN) required for clearing scar tissue and induces the secretion of pro-angiogenic molecules like VEGF, angiogenin and PLGF. Moreover, DHT activates protein pools involved in signaling following injury, metabolic processes linked to wound healing.

Apart for conducting thorough in-depth experiments to address his thesis goals, Mr. Popa has also shown his innovative skills and patented a method for cell migration assay. The data presented in this dissertation is of scientific importance and contributes importantly to the field of regenerative medicine. In my opinion, the work presented in this dissertation is novel and scientifically adequate for consideration for a PhD degree. As a specialist member of the Doctoral Commission approved by the SCOSAR Scientific Council Decision no. 5200/29.11.2018, I approve and recommend this dissertation by Mr. Popa Mirel Adrian for a PhD degree without any reservation.

Raghvendra K Dubey, PhD;

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Of the Romanian Cell Academy

Institute of Biology and Pathology
"Nicolae Simionescu"

PHD THESIS SUMMARY

with the title

Effect of androgenic hormones on mesenchymal stromal / progenitor cells involved in cardiovascular regeneration

Defended on 19/12/2018,

George Emil Palade Hall of Institute of Cellular Biology and Pathology "Nicolae Simionescu"

Coordinator: Acad. Maya Simionescu

PhD student: Popa Mirel Adrian

The doctoral thesis can be consulted at

Institute of Cell Biology and Pathology "Nicolae Simionescu"

CONTENTS

CURRENT STATE OF KNOWLEDGE

PERSONAL CONTRIBUTIONS

I. Studies on the effect of dihydrotestosterone on proliferation, migration and integration of MSC into murine ventricular sections

II. Research on the effect of dihydrotestosterone on proliferation, migration and integration of human progenitor endothelial cells into murine ventricular sections

SUMMARY

Keywords: mesenchymal stromal cells, endothelial progenitor cells, androgen, dihydrotestosterone, androgen receptor, cell therapy, cardiovascular diseases, placental blood, Wharton's jelly, murine ventricular sections.

Total number of pages - 165

Number of figures in the "Actual Stage of Knowledge" part - 11

Number of figures in the "Original contributions" section - 31

Number of tables - 5

Bibliographic indications - 326

Works published in international journals indexed ISI - 3 (1 first author)

Works published in international journals indexed BD I -1 (1 first author)

National patents - 2

Summaries published in ISI - 1 indexed journals

Preparatory work - 2

Oral communications held at scientific events international - 7 (2 first author)

Posters presented at scientific events international - 1 4 (13 first author)

Posters presented at scientific events national - 1 (co-author)

Communications hours held at scientific events national - 1 (co-author)

Courses and specializations - 2

National Awards - 1

Scholarships obtained during the doctoral program - 2

Participation in international / national research projects - 4

Cardiovascular diseases are the leading causes of worldwide mortality. Although, is the core of a considerable number of studies, myocardial infarction remains one of the major fatal cardiovascular disorders resulting from obstruction of coronary arteries and cardiomyocyte destruction.

Diseases of the cardiovascular system affect the heart and blood vessels (arteries, veins and capillaries). With aging, physiological and morphological changes can alter the functions of the cardiovascular system and the risk of cardiovascular disease increases dramatically. The majority of diseases and deaths due to cardiovascular disease have as their primary triggering factor atherosclerosis. This process begins with the thickening and stiffening of the arterial wall due to cholesterol deposition in the vessels walls, thereby forming atheromatous plaques, which results in obstruction of blood flow. Atherosclerosis affects all the arteries in the body. The onset of atherosclerosis in the coronary arteries is an early one, this disease being called coronary disease, which can cause acute myocardial infarction (AMI) at the advanced stage of development. AMI is the result of obstructing blood flow, thereby reducing the oxygen supply to the myocardium, ultimately resulting in tissue damage. The affected area becomes hypoxic, triggering inflammatory processes and subsequently regeneration. Heart remodeling is final by scarring the damaged tissue.

Recent advances in stem cell research for tissue regeneration highlight the potential use of pluripotent/precursor adult stem cells for the reconstruction or regeneration of different tissues without the inclusion of ethical issues (Nesselmann *et al.,* 2008, Karantalis & Hare 2015, Singh *et al.,* 2016a). In this context, multipotent mesenchymal stromal cells (MSC) that are capable of transforming into different cell types have gained attention to serve as a therapeutic tool for permanent repair/regeneration of damaged cardiac tissue. MSC are of mesodermal origin and can be differentiated into different types of cells (endothelial cells, osteoblasts, chondrocytes, adipocytes, myocytes) when exposed to specific growth factors, signaling molecules and transcription factors (Karantalis & Hare 2015).

More importantly, they can be grown and prepared for *in vitro* delivery (Karantalis & Hare 2015). MSC can be isolated from multiple sources (fat, placenta, intestine, lung, liver, amniotic fluid, dental pulp, periodontal pulp, heart). Together, multiple tissue sources and the pluripotent nature of MSC in sync with ease of *in vitro* expansion make them a curative therapeutic tool for treating cardiac lesions.

Over the past decade, several clinical trials have been observed in both animals and humans, assessing the therapeutic potential of MSC in repairing cardiac lesions and functions (Nesselmann*et al.,* 2008, Singh *et al.,* 2016b). Although many studies (not all) have been established in which MSC therapy can lead to a potential improvement in neovascularization, scarring and functional recovery, a major barrier to MSCs therapy is the low retention of transplanted cells, ie 0.44% of transplanted MSC are found in the myocardium after 4 days (Toma *et al.,* 2002; Müller-Ehmsen *et al.,* 2006). This has led to a demand for further optimization of MSC therapy by identifying molecules that could be used to receive/treat MSCs or recipients to increase MSC's ability to regenerate the ischemic area (Novotny *et al.,*2008). Since it has been shown that androgens stimulate growth influences cell activity cardiovascular (Yeap *et al.* 2003; Ikeda *et al.* 2005), promotes the growth and angiogenesis in endothelial progenitor cells (EPC) and mesenchymal stromal cells (Cai *et al.* 2016), we assume that androgen can also facilitate the adhesion and integration into the ischemic tissue of both MSC and EPC.

Endothelial cells (EC) are needed for tissue repair and wound healing by building a new blood vessel network. Interestingly, in addition to residual EC, circulating EPCs derived from bone marrow and spleen were found to be actively involved in vascular repair. Therefore, our main focus was also studying the effects of androgen on the EPC function. The relationship between testosterone deficiency and the reduced number of circulating EPCs is a hallmark of cardiovascular disorders, as demonstrated in hypogonadal patients (Foresta et al., 2006, Calogero et al., 2017).

There are plenty of evidences suggesting a central role of androgen in male cardiovascular health. Despite numerous studies to decipher the precise roles of testosterone (T) in cardiovascular disease (CVD), no agreement has been reached on the protective or harmful effects of T on the cardiovascular system (CVS). It is therefore essential to clarify how T affects CVS in order to identify the potential risks and benefits of testosterone replacement therapy (TST), increasingly used by older men.

We assume that androgens also modulate vascular remodeling processes associated with CVD. In the foreground, there is the ability of MSC and EPC to migrate, the pro-angiogenic responsiveness of both cell types and, last but not least, the chemokines activated in response to androgen pre-treatment. All of these processes are aimed at improving cardiovascular ischemia regeneration capacity.

To answer these questions, we used dihydrotestosterone (DHT), a T-metabolite that mimics the effects of T and cannot be converted to estradiol. Therefore, DHT use can certify that the observed effects are only androgenic and are not compromised by conversion to estradiol.

Knowing all of the above, we have delivered the potent basis of this study, namely: it is possible to enhance the capacity of MSC and EPC to adhere, integrate and proliferate by pre-stimulation with androgenic hormones in such a way that chances of ischemic regeneration cardiovascular to be improved?

In the **first part** of the doctoral dissertation are briefly described the theoretical aspects related to the structure and actions of androgenic hormones, characteristics and properties of mesenchymal stromal cells and progenitor endothelial cells, up-to-date aspects of androgen hormones and stromal/progenitor cells in the treatment of cardiovascular diseases. The **second part** of the thesis, which includes the original contributions of this doctoral thesis, is divided into two major studies.

The first study covered **"Studies on the effect of dihydrotestosterone on the proliferation, migration and integration of MSC in murine ventricular slices"** and is characterized by two general objectives:

- investigating the effects of DHT on the proliferation, migration and adhesion of MSC to murine myocardium;
- and assessing the role of AR in the modulating effects of DHT on MSC functions.

The second study refers to "**Research on the effect of dihydrotestosterone on proliferation, migration and integration of human progenitor endothelial cells in murine ventricular sections**" and is divided into two parts:

- determining the molecules that can mediate DHT-induced effects on EPC migration/ adhesion functions in the murine myocardium;
- evaluating the interaction between AR and EPC activity after pre-treatment with DHT.

To meet all the objectives of this PhD thesis, we have used various techniques of biochemistry, molecular biology and cell cultures that have been described in detail in the section "Materials and Methods" characteristic of each chapter. For better reproducibility of the results obtained, the protocol for obtaining the mouse heart sections was optimized together with the adhesion of the heart sections on the bottom culture wells. A patented method has been developed, that allowed us to measure in real-time the cells migration based on cellular impedance, using the xCELLigence device. Also, a method was optimized for quantifying human cells in the direct co-culture model between MSC or EPC and the murine heart slices using the qRT-PCR technique.

Increased life expectancy is also accompanied by increased demand for improved quality of life and prevention of aging diseases. Since testosterone deficiency was suggested to be associated with CVD, the number of men undergoing testosterone supplementation (TST) increased. However, some studies have reported adverse effects of TST and increased mortality due to CVD. Therefore, it is necessary to understand the role of T in cardiovascular health and if TST is beneficial. The role of EPC in cardiovascular regeneration is well established and is currently being used in cardiovascular therapy. In hypogonadal men, it has been shown that lower levels of EPC are directly correlated with the predisposition of cardiovascular disease development.

Consistent with these findings, the original results show that the hypothesis issued in the present study were correct as follows:

- DHT increases the proliferation and ability of MSC to adhere to cardiac tissue;
- In MSC, the proteins and genes expression of MMP-2, MMP-9 and EMMPRIN is modulated by DHT;
- DHT increases migration and chemotaxis of MSC to cardiac tissue;
- The secretion of angiogenin, VEGF and NO - key factors in the process of ischemic tissue regeneration is increased in DHT-stimulated MSC;
- MSC cytoskeleton proteins involved in cell migration are activated following stimulation with DHT;
- DHT induces increased proliferation, migration and EPC's ability to form capillary networks on Matrigel® substrate in an AR-dependent manner;

- Proteolytic key molecules (EMMPRIN and MMP-9) are also upregulated by DHT treatment;
- The secretion of angiogenin, VEGF and PLGF - key factors involved in the onset and development of regenerative processes by angiogenesis is increased in DHT-stimulated EPC;
- By stimulating with DHT the EPC activates large protein groups that are involved in signaling the response to injury, wound healing, the metabolic process of the organonitrogen compound.

The main results of the first part of this thesis build up the role of DHT in the growth, proliferation and migration of MSC and in promoting the integration of these cells in cardiac tissue *in vitro*. These effects are accompanied by an increase in AR expression and key molecules known to promote tissue remodeling and angiogenesis (EMMPRIN, MMP-2, MMP-9, VEGF, angiogenin, NO). These original data contained in the **first study** of this doctoral thesis were reflected in the article entitled: "Dihydrotestosterone induces pro-angiogenic factors and assists homing of MSC into the cardiac tissue ", has as authors: Popa MA * , Mihai MC * , Constantin A, Șuică V, Țucureanu C, Costache R, Antohe F, Dubey RK & Simionescu M (* equal contribution) and published in 2018 in the *Journal of Molecular Endocrinology* 60 1-15. (doi: 10.1530 / JME-17-0185) (Impact factor 2017 IF= 3.29).

The main data from the **second part** deciphers another role of DHT following EPC stimulation. We have shown that DHT regulates the EPC function of forming capillary tube -like structures by increasing the number of closed structures. We have shown that AR is involved in cell migration processes in the presence of DHT. Moreover, we have shown that DHT regulated the expression of proteins involved in cell migration, EMMPRIN and MMP-9, and the expression of pro-angiogenic proteins is increased due to the presence of DHT (VEGF, VEGFR-2, angiogenin and PlGF). We assume that DHT also activates the PI3-k/AKT signaling pathway through AR, a pathway that enhances EPC proliferation and cellular proliferation (Liu et al., 2014). Therefore, our research supports the concept of the protective effects of androgen on EPC function by stimulating the formation of new blood vessels and improving the endothelial integrity. This original data is part of an article to be published.

The new data from our experiments indicates that *in vitro* DHT stimulation of MSC and EPC retains cell properties and significantly increases adhesion and cell proliferation. However, additional studies are needed to determine the mechanisms involved in the effect of DHT on the survival, mobilization and stimulation of MSC and EPC. Increasing the proliferation potential of MSC and EPC stimulated with DHT without affecting the potential for action could make these cells not only a simple candidate, but an excellent option for vascular regenerative medicine and ischemic illnesses in general.

Perspectives

The therapeutic use of mesenchymal stromal cells and progenitor endothelial cells in the treatment of cardiovascular lesions has not yet reached its potential due to the reduced efficiency of grafting in the damaged tissue but also to the deficient mechanisms of the host organism. In this paper we have demonstrated that pre-treatment of MSC and EPC with DHT improves cellular migration capacity in response to cardiac stimuli and also increases the pro-angiogenic profile of stimulated cells.

A future project would be based on experiments to investigate the mechanisms and effects of DHT on co-culture between MSC and EPC co-cultivated in the presence of murine sections. Since the proteolytic activity of cells is stimulated by the presence of DHT, it is also noticeable how the set of pro-inflammatory cytokines in the onset of post-ischemic fibrosis is affected.

An intermediate step of the future project is the use of DHT-stimulated cells in tissue engineering as a support for *ex vivo* grafts. Knowing that cells behave differently in a two-dimensional construct, analyzing the functions and molecules activated by three-dimensional cultivation can reveal new functions. The emphasis will be placed on the proteolytic activity of MMP and the synthesis of pro-angiogenic factors to which the set of vascular ischemia-specific immune modulators will be added.

Before proceeding to *in vivo* experiments, all of the data will be added as a variable in an *in silico* simulation model which will be based on artificial intelligence algorithms. These *in silico* algorithms will be structured in the form of databases and neural networks that will simulate possible ways of action by the molecules of interest based on probabilities. This analysis aims at streamlining *in vivo* studies and minimizing confirmatory experiments on animal subjects.

WORKS PUBLISHED DURING THE DOCTORAL PROGRAM

❖ Works published in ISI indexed journals - first author

1. **Popa MA** *, Mihai MC *, Constantin A, Șuică V, Țucureanu C, Costache R, Antohe F, Dubey RK & Simionescu M, 2018. „Dihydrotestosterone induces pro-angiogenic factors and assists homing of MSC into the cardiac tissue". *Journal of Molecular Endocrinology* 60 1-15. (doi: 10.1530 / JME-17-0185) (* equal contribution) (IF in 2017 = 3.29).

❖ Works published in ISI indexed journals - co-author

1. Corotchi MC *, **Popa MA** *, Remeș A, Sima LE, Gussi I & Lupu Plesu M, 2013. "Isolation method and xeno-free culture conditions influence multipotent differentiation capacity of human Wharton's jelly-derived mesenchymal stem cells". *Stem Cell Research and Therapy* 4 81. (doi: 10.1186 / scrt232); (* equal contribution) (IF in 2017 = 4.963).

1. Corotchi MC, **Popa MA**, Simionescu M, 2016. „Testosterone stimulates proliferation and preserves stemness of human adult mesenchymal stem cells and endothelial progenitor cells". *Journal of Romanian Morphology & Embryology* 57 75-80. (doi: 10.1093 / bioinformatics / btl627). (IF in 2017 = 0.811).

❖ Works published in magazines indexed by BDI - first author

1. **Popa MA** * & Corotchi MC *, 2015. „An in vitro method for adhesion of fresh adult murine heart slices on Collagen - Coated surfaces". *Annals of the Romanian Society for Cell Biology* 20 35-39. (doi: 10.ANN / RSCB-2015-0044: RSCB).

❖ Summaries published in ISI indexed journals

1. Corotchi MC* & **Popa MA***, 2015. „Effects of 17- β estradiol in a novel co- culture system of human Wharton's jelly-derived mesenchymal stem cells with adult- heart murine ventricular slices on cardiac regeneration". *Atherosclerosis* 241 e93. (doi:10.1016/j. atherosclerosis.2015.04.326) (* equal contribution).

❖ Works under publication

1. **Popa MA***, Mihai (Corotchi) MC*, Constantine, Șuică V, Țucureanu C, Costache R Antohe M, Dubey RK Simionescu M. "The effect of dihydrotestosterone on proliferation, migration and integration of human progenitor endothelial cells in murine ventricular sections " (* equal contribution);

2. Maria Cristina Mihai (Corotchi) *, **Mirel Adrian Popa** *, Viorel Iulian Șuică, Felicia Antohe, Edwin K Jackson, Maya Simionescu, Raghvendra K Dubey. " Mechanism of 17- β Estradiol stimulated integration of human mesenchymal stem cells in heart tissue ", (* equal contribution).

❖ **National patents**

1. "Device for Modifying Cell Culture Plate, and Real-Time Measurement of Cell Movement in *Ex vivo* System " - No. 131463/2018;
2. "Ex-vivo Procedure for Grafting Progenitor Endothelial Cells in the Adult Myocardium" - No. 162258/2018.

ORAL COMMUNICATIONS SUPPORTED TO INTERNATIONAL SCIENTIFIC MANIFESTATIONS - MAIN AUTHOR

1. Maria Cristina Corotchi *, **Mirel Adrian Popa** *, Anca Remeş, Livia Elena Sima, Ilinca Gussi, Marilena Plesu (Lupu) (* equal contribution). " Isolation method and xeno-free culture conditions influence multipotent differentiation capacity of human Wharton's jelly-derived mesenchymal stem cells ", RAMSES Final Conference, 7-9 May 2013, Cairo, Egypt.
2. **Mirel Adrian Popa** *, Maria Cristina Corotchi*, Maya Simionescu (* equal contribution). "DHT enhance migration and integration of mesenchymal stem cells towards heart tissue sections ". The 9th International Congress and the 35th Annual Scientific Session of the Romanian Society for Cell Biology, 7-11 June 2017, Iasi, Romania.

POSTERS PRESENTED IN INTERNATIONAL SCIENTIFIC MANIFESTATIONS - MAIN AUTHOR

1. **Mirel Adrian Popa***, Anca Remes*, Maria-Cristina Corotchi, Marilena Lupu (* equal contribution). "Gene and protein profiling of human Wharton's Jelly-derived stem / progenitor cells upon exposure to endothelial differentiation conditions ", The 4th International Congress and the 30th Annual Scientific Session of the Romanian Society for Cell Biology, 13-17 June 2012, Satu-Mare, Romania and Debrecen, Hungary takes; a summary published in the Bulletin of the National Society of Cell Biology, No 40, p. 152.
2. Maria Cristina Corotchi *, **Mirel Adrian Popa** *, Anca Remeş, Livia Elena Sima, Ilinca Gussi, Marilena Plesu (Lupu) (* equal contribution). " Isolation method and xeno-free culture conditions influence multipotent differentiation capacity of human Wharton's jelly-derived mesenchymal stem cells ", RAMSES Final Conference, 7-9 May 2013, Cairo, Egypt.
3. Maria Cristina Corotchi *, **Mirel Adrian Popa** *, Anca Remeş, Livia Elena Sima, Ilinca Gussi, Marilena Plesu (Lupu) (* equal contribution). " Isolation method and xeno-free

culture conditions influence multipotent differentiation capacity of human Wharton's jelly-derived mesenchymal stem cells ", The 5th International Congress and the 31st Annual Scientific Session of the Romanian Society for Cell Biology, 5-9 June 2013, Timisoara, Romania; a summary published in the Bulletin of the National Society of Cell Biology, no. 41, p. 122.

4. **Mirel Adrian Popa,** Maria Cristina Corotchi, Anca Remeş, Marilena Lupu. " Endothelial gene and protein expression in human Wharton's jelly-derived stem / progenitor cells isolated in different conditions", 7th Annual Congress of the German Society for Stem Cell Research, 28-30 October 2013, Leipzig, Germany.

5. **Mirel Adrian Popa** *, Maria Cristina Corotchi *, Maya Simionescu (* equal contribution). "Impact of dihydrotestosterone on human postnatal cord blood and matrix derived adult stem / progenitor cells " The 6th International Congress and the 32nd Annual Scientific Session of Romanian Society for Cell Biology, 7-12 June 2014, Târgu Mureş, Romania; a summary published in the Bulletin of the National Society of Cell Biology, no. 42, p. 65.

6. Maria-Cristina Corotchi *, **Mirel Adrian Popa** *, Maya Simionescu (* equal contribution). " Effects of 17- β estradiol on proliferation and cardiac integration of human Wharton's Jelly-derived mesenchymal stem cells ". The 6th International Congress and the 32nd Annual Scientific Session of the Romanian Society for Cell Biology, 7-12 June 2014, Târgu Mureş, Romania; a summary published in the Bulletin of the National Society of Cell Biology, no. 42, p. 67.

7. **Mirel Adrian Popa** *, Maria Cristina Corotchi *, Maya Simionescu (* equal contribution). " Effect of dihydrotestosterone on adult stem cells derived from human postnatal cord blood and matrix". 7th Santorini Conference "Biology Prospective" Systems Medicine, Personalized Health and Therapy, 25-27 September 2014, Santorini, Greece.

8. Maria Cristina Corotchi*, **Mirel Adrian Popa***, Maya Simionescu (* equal contribution). " Chemo-attraction of human Wharton's jelly-derived mesenchymal stem cells mediated by 17- β estradiol in cardiac integration " The 7th International Congress and the 33rd Annual Scientific Session of the Romanian Society for Cell Biology, 11-14 June 2015, Baia Mare, Romania; a summary published in the Bulletin of the National Society of Cell Biology, No 43, p. 104.

9. Maria Cristina Corotchi*, **Mirel Adrian Popa***, Maya Simionescu (* equal contribution). "Testosterone exposure enhances proliferation, adhesion and viability and preserves stemness of human stem cells " 41st WORLD CONGRESS OF ISMH - May 19-21, 2016, Bucharest, Romania.

10. **Mirel-Adrian Popa** *, Maria Cristina Mihai * (* equal contribution). "In vitro adhesion of fresh adult murine heart slices on collagen-coated surfaces " 41st WORLD CONGRESS OF ISMH - 19 to 21 May 2016, Bucharest, Romania.

11. **Mirel Adrian Popa** *, Maria Cristina Corotchi *, Maya Simionescu (* equal contribution). " MMPs are involved in the migration and chemotaxis of DHT- stimulated mesenchymal stem cells towards heart tissue sections ". The 8th International Congress and the 34th Annual Scientific Session of the Romanian Society for Cell Biology, 8-12 June 2016, Băile Felix, Romania; a summary published in the Bulletin of the National Society of Cell Biology, no. 44, p. 104.

12. **Mirel-Adrian Popa** *, Maria-Cristina Mihai * , Alina Constantin, Viorel Şuică, Raluca Costache, Felicia Antohe, Raghvendra K Dubey, Maya Simionescu (* equal contribution). "Human mesenchymal stem cells migration proteins is upregulated by dihydrotestosterone treatment ". 12th CEEPC 2018. 24-26 October, Bucharest, Romania.

ORAL COMMUNICATIONS SUPPORTED TO INTERNATIONAL SCIENTIFIC MANIFESTATIONS - CO-AUTHOR

1. Marilena Lupu, Anca Remeş, Maria Cristina Corotchi, **Mirel Adrian Popa**, Maya Simionescu, "Cellular and molecular mechanisms of umbilical cord stem/ progenitor cells induced vasculogenesis - implications for cardiovascular regeneration ", The 4th International Congress and the 30th Annual Scientific Session of Romanian Society for Cell Biology, June 13-17, 2012, Satu-Mare, Romania and Debrecen, Hungary.

ISSUES PRESENTED IN INTERNATIONAL SCIENTIFIC MANIFESTATIONS - CO-AUTHOR

1. Maria Cristina Corotchi *, Anca Remeş *, **Mirel Adrian Popa**, Livia Sima, Marilena Lupu (* equal contribution). " Characterization of multipotent differentiation capacity of human Wharton's jelly-derived mesenchymal stem cells isolated and expanded in xenobiotic-free Conditions " The 4th International Congress and the 30th Annual Scientific Session of Romanian Society for Cell Biology, 13-17 June 2012, Satu-Mare, Romania and Debrecen, Hungary; a summary published in the Bulletin of the National Society of Cell Biology, no. 40, p. 151;

2. Maria Cristina Corotchi, Anca Remeş, **Mirel Adrian Popa**, Livia Sima, Marilena Lupu. " Xenobiotic-free Conditions Applied for in vitro manipulation of Wharton's jelly-derived mesenchymal stem cells, "7th Annual Congress of the German Society for Stem Cell Research ", 28 to 30 October 2013, Leipzig, Germany;

3. Ciprian Neagoe, **Mirel Adrian Popa**. " Numerical simulation of leukocyte adhesion to endovascular surfaces ". 1st International Conference on Emerging Technologies in

Materials Engineering EmergeMAT and 4th International Workshop on Materials under Extreme Conditions SUPERMAT. 14-16 November 2018, Bucharest, Romania.

ORAL COMMUNICATIONS AND POSTERS SUPPORTED TO NATIONAL SCIENTIFIC MANIFESTATIONS - MAIN AUTHOR AND CO-AUTHOR

1. Maria Cristina Corotchi, **Mirel Adrian Popa**, Marilena Lupu. "Characterization of multipotent differentiation capacity of human Wharton's jelly-derived mesenchymal stem cells isolated and expanded in xenobiotic-free conditions ", RAMSES Annual International Workshop, 13-16 September 2012, Nicolae Simionescu Institute of Cell Biology and Pathology, Bucharest, Romania.

SPECIALIZATIONS AND COURSES PERFORMED

- Congress "*The first international society of regenerative medicine and surgery congress. New frontiers in regenerative medicine and surgery. Interdisciplinarity, Research and Clinical Applications* ", Bucharest, Romania, May 15 - 16, 2015;

- The International Congress of Regenerative Medicine. Regenerative medicine; research and clinical applications, Bucharest, Romania, **14-17** June **2017.**

NATIONAL PRIZES OBTAINED

- Best Poster Award (1st place) - **Mirel Adrian Popa***, Anca Remeș*, Maria Cristina Corotchi, Marilena Lupu (* equal contribution). "*Gene and protein profiling of human Wharton's Jelly-derived stem / progenitor cells upon exposure to Endothelial Differentiation Conditions* ", The 4th International Congress and the 30th Annual Scientific Session of the Romanian Society for Cell Biology, 13-17 June 2012, Satu-Mare, Romania and Debrecen, Hungary;

- Best Poster Award (1st place) - Maria Cristina Corotchi *, Anca Remeș *, **Mirel Adrian Popa**, Livia Sima, Marilena Lupu (* equal contribution). " Characterization of multipotent differentiation capacity of human Wharton's jelly-derived mesenchymal stem cells isolated and expanded in xenobiotic-free Conditions " The 4th International Congress and the 30th Annual Scientific Session of Romanian Society for Cell Biology, 13-17 June 2012, Satu-Mare, Romania and Debrecen, Hungary; a summary published in the Bulletin of the National Society of Cell Biology, no. 40, p. 151.

- National patent No. 131463/2018 awarded by UEFISCDI (Executive Unit for Financing Higher Education, Research, Development and Innovation)

SCHOLARSHIPS BETWEEN DOCTORAL DURATION AND FINANCING OF RESEARCH

- ✓ The Romanian Academy through SCOSAR (2013-2016);
- ✓ POSDRU / 159 / 1.5 / s / 133391 "Doctoral and post-doctoral programs of excellence for the training of highly qualified human resources for research in the fields of Life Sciences, Environment and Earth "(April 2014 - December 2015)

❖ **National and international grants collaborator:**

1. „*Ramses: Reinforcement of the Adult Stem cell research area through Mobility and Scientific networking between Egypt, Romania and a German Consortium for Strengthening the international scientific competence*". Grant agreement no. 245691 (November 2011 - August 2013);
2. „*Investigation of molecular mechanisms of integration and vasculogenesis of umbilical cord blood stem cells; implications for cardiovascular regeneration",* acronym" RE-CORD ", PNCDI-II type TE (November 2011 - June 2013);
3. *„Effects of sex steroid son adult stem / progenitor cell mediated cardiovascular regeneration"*, Romanian- Swiss Research Program (January 2013 - June 2016)
4. „*Improving institutional competitiveness in the field of type 1 diabetes by developing an innovative concept of mesenchymal stromal cell immunotherapy – DIABETER"* (April 2018 - present).

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